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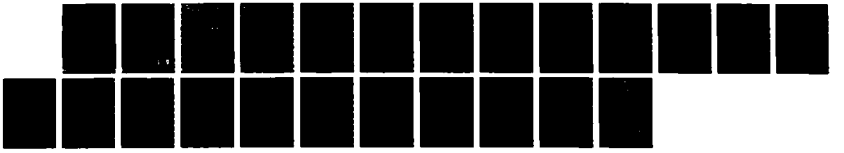

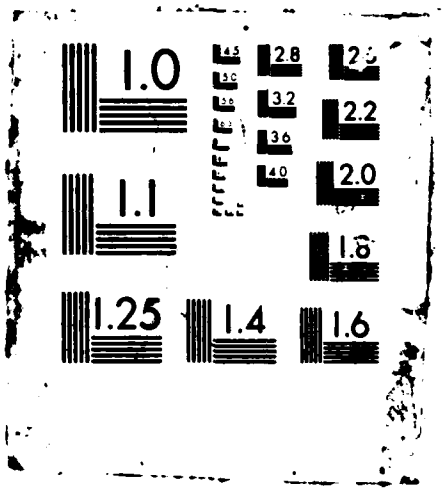
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LASSA FEVER IMMUNE PLASMA

ANNUAL SUMMARY REPORT

John D. Frame, M.D.

July 31, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrdick, Frederick Maryland 21701-5012

Contract No. DAMD17-85-C-5189

Columbia University College of Physicians and Surgeons
630 W. 168th Street
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The bulk of clinical investigation was carried out at CLH and PH. Twenty-four cases of LF and four of possible LF (PLF) were diagnosed among 120 febrile patients treated at CLH, and seven cases of LF and three of PLF among 252 patients at PH.

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Appropriate investigation of the efficacy of LFIP and ribavirin will require more rapid diagnosis than is at present possible in Liberia. It is hoped that rapid diagnosis will be achieved with the use of enzyme-linked immunosorbent assay for viral antigen which has been developed already, and which will likely be field-tested in Liberia in the coming year.

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Summary

Investigations of Lassa fever (LF) in Liberia continued in the first year under contract DAMD17-85-C-5189. The ultimate objectives of the program include the collection of Lassa Fever Immune Plasma (LFIP) units and forwarding the majority of the plasma to the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), and ultimately, therapeutic trials of the plasma and comparison of its effectiveness with ribavirin, an antiviral agent.

Plasmapheresis was conducted at Curran Lutheran Hospital (CLH), and increasingly at Phebe Hospital (PH) with 255 units collected in all; of these, 189 were forwarded to USAMRIID. Three donors were found to be carriers of Hepatitis B surface antigen (HBsAg) and will be excluded from future donations. An LFIP unit from one HBsAg-positive donor was forwarded to USAMRIID before the presence of the antigen was noted, and will be discarded there.

The bulk of clinical investigation was carried out at CLH and PH. Twenty-four cases of LF and four of possible LF (PLF) were diagnosed among 120 febrile patients treated at CLH, and seven cases of LF and three of PLF among 252 patients at PH.

Passive immunotherapy was attempted in both hospitals. Three of six confirmed LF patients treated at CLH expired; they were all pregnant. At PH two of the six patients treated were subsequently found to have had bacterial sepsis rather than LF; one died. The other four cases survived; none was confirmed to have LF by serological tests, and virus isolation was not attempted in any, so that the diagnosis remains in doubt in all these instances.

Appropriate investigation of the efficacy of LFIP and ribavirin will require more rapid diagnosis than is at present possible in Liberia. It is hoped that rapid diagnosis will be achieved with the use of an enzyme-linked immunosorbent assay for viral antigen which has been developed already, and which will likely be field-tested in Liberia in the coming year.

FOREWARD

For the protection of human subjects the investigatos have adhered to policies of applicable Federal Law 45CFR46.

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Introduction

The present investigation follows earlier work with Lassa fever (LF) in Liberia. During this time the incidence of LF in several Liberian hospitals, the prevalence of Lassa virus antibodies (LVA) and the collection of Lassa fever immune plasma (LFIP) units indicated that intensive and controlled therapeutic trials might be productive.

Lassa Fever was first recognized in Liberia in 1972 in an outbreak at the Curran Lutheran Hospital (CLH) in which 11 cases were identified. Four of them were among hospital staff members, and resulted in four deaths, including two in the staff, one of them an American nurse (1,2). Subsequent investigations demonstrated a high prevalence of LVA among hospitals staff members throughout Liberia (3,4), and that LF was the cause of between 10 and 20% of illnesses among febrile patients admitted to several hospitals in northwest Liberia (5). A group of convalescents was found willing to accept plasmapheresis and donate plasma, and LFIP units were obtained. Many were sent to the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), and some were retained in Liberia for treatment of patients there.

During the course of investigations at USAMRIID it was found that high titers of neutralizing antibodies (NA) did not develop until several months after the onset of infection (6). After the first few months of plasmapheresis in Liberia convalescents were approached for donation 6 months or more after their recovery from LF. A relatively high proportion of donors were found to have titers of NA as defined by the Log Neutralization Index (LNI) high enough to be considered therapeutic.

Therapeutic trials of LFIP were not conclusive. Because of the limited amount of plasma available only those patients considered to be the most seriously ill were treated, so that there were no appropriately randomized controls. There did appear to be a beneficial effect in some patients in whom plasma infusion was followed by sudden improvement in the clinical status, an outcome noted as well in some other clinical studies of LFIP (7,8).

In addition to the limited supply of plasma, two other factors confounded attempts to investigate the efficacy of LFIP. In Liberia LF has a relatively low mortality of about 5% among non-pregnant adults, so that a very large number of patients would have to be assembled in order to demonstrate the value of treatment as compared with no treatment, if survival of the patients were to be the criterion of success. The clinical course of LF in Liberia is too variable to permit clear-cut determinations of efficacy to be made from the course of illness alone. The other factor was that the laboratory diagnosis of LF was being made too late to permit treatment of all and only LF cases. Serological diagnosis can only be made by comparison of titers of specimens taken over the course of several days, and virus isolation can only be performed in a

containment laboratory such as that at USAMRIID. Some cases are missed by clinical diagnosis alone, and other fevers, not yet clearly defined, often lead to immunotherapy of patients later found not to have LF.

The solution to the first question, that of a criterion for determination of therapeutic success, may be solved by measurement of the LV viremia during the course of treatment. In the course of the earlier investigations, ultrafreezers were installed in the hospitals where treatment will be undertaken, and it is hoped that virus activity will be maintained to a degree sufficient to permit the comparison of virus titers before and after treatment. The issue of rapid diagnosis will likely be solved by the development of an enzyme-linked immunosorbent assay (ELISA) (9) that will permit diagnosis within hours of the patient's admission to the hospital.

Another potential therapeutic modality is the use of ribavirin, an antiviral agent which has been found successful in the treatment of LV infections in monkeys (10) and in humans (11). Thus, in response to a request for a proposal from the U.S. Army Medical Research and Development Command (DAMD17-84-R-0099, issue date: 1 Aug 1984) we proposed an investigation of the comparison of LFIP and ribavirin in the treatment of LF, and contract DAMD17-85-C-5189 was awarded to permit investigations to this end.

Activities

Management and most of the conduct of the investigations in Liberia are the responsibility of Mr. J. E. Yalley-Ogunro, Field Investigator and Resident Head of the Lassa Fever Control Project of the Liberian Institute for Biomedical Research (LIBR). In August, 1985, he traveled to CLH to conduct plasmapheresis, and returned for the same purpose in October and December, 1985, and March, May and June, 1986. In October and December, 1985, and in April and June, 1986, he traveled to the other field station at Phebe Hospital to conduct plasmapheresis. At both sites he conferred with the laboratory workers who are part of the investigative team, reviewed their records and checked their procedures, and returned to the LIBR with patient sera they had collected.

At the LIBR Mr. Yalley-Ogunro tested the sera by means of the indirect fluorescent antibody (IFA) test. For much of the year he was unable to carry out testing because of difficulties with the spot antigen slides supplied to him by USAMRIID. It appears that the commercial supplier of the slides on which LV antigen in cell culture is deposited had furnished USAMRIID faulty slides. New antigen prepared on slides from a different supplier was sent to him in May, and he then was able to conduct the tests which had been delayed for most of the year.

During a vacation trip to the United Kingdom and the United States in December and January Mr. Yalley-Ogunro visited USAMRIID for 3 days. When the investigators there had seen his competence,

USAMRIID requested that we request funds to permit him to work there for 3 to 6 months during the summer. Because of limitations of funds, and the need for Mr. Yalley-Ogunro's services in Liberia, the period at USAMRIID is being limited to 3 months. During this time, he is to become familiar with the ELISA test and carry out several serological studies of sera which have been stored there to assist in elucidating some of the other causes of febrile illness occurring in northwest Liberia, fevers that confound the diagnosis of LF.

Andrew K. Cole, M.D., lives in the town of Kolahun about 100 miles to the northwest of Zorzor, where CLH is located. Dr. Cole has been working with Mr. Yalley-Ogunro to determine the prevalence of LV infections in villages in that region (11,12). He is responsible for supervision of the Tellewoyan Memorial Hospital in Voinjama, the county seat of Lofa County in which Zorzor and Kolahun are located. He is instructing the staff in the diagnosis of LF and is preparing for investigations to be conducted in that hospital, once adequate refrigeration is available. A goal of his during the remainder of the project will be to determine the incidence of LF in the region about Kolahun, an area which will be appropriate for field studies of a LV vaccine when one is prepared.

Dr. Mark H. Monson, Chief Medical Officer at CLH, is not funded under the Contract. However, he furnishes invaluable help to the clinical aspects of the investigation, and will be responsible for actual patient management during the therapeutic trials. He was on leave in the United States, but returned to CLH in September, 1985, and has been reviewing the clinical experience accumulated during his absence.

The principal investigator, Dr. John D. Frame, visited Liberia in October, 1985, and May, 1986. Much of his time is spent in coordinating the activities in the field with the program at USAMRIID. In November he participated as a member of the Test Integration Working Group of the U.S.Army Medical Materiel Development Activity for the Lassa Fever Immune Plasma Program, which discussed topics pertinent to submission of an IND for Lassa Immune Plasma for Globulin, proposed clinical testing, laboratory evaluation, and other issues.

In Liberia he consults with Dr. Aloysius Hanson, Director of the LIBR, on matters involving the project there. He also instructs laboratory and medical workers in the Liberian hospitals, and after evaluating the status of work, helps define new goals for the investigations and treatment of LV infections under the terms of the contract.

Plasmapheresis

Plasmapheresis was conducted during six trips to CLH and four to PH. For some time attempts had been made to obtain reliable testing for Hepatitis B surface antigen (HBsAg), and results finally came to hand during the year; three donors were found antigenemic.

Except for one LFIP unit fowarded before this information was at hand, none of their plasma was sent to USAMRIID. The results of testing for Human Immunodeficiency Virus (HIV) were also completed; none of our donors was found positive for this agent. As in former years attempts were made to forward to USAMRIID only LFIP units with a Log Neutralization Index (LNI) of at least 0.3. During the latter part of 1985, some testing of donor specimens for neutralizing antibodies (NA) was delayed because of the absence of Dr. Peter Jahrling from USAMRIID. It was not always feasible to postpone plasmapheresis or shipment of plasma until the results of tests were at hand.

Two hundred fifty-five units of convalescent plasma were obtained by plasmapheresis, and 189 forwarded to USAMRIID. Of these, one was HBsAg positive. Until testing for the LNI of the remaining units has been completed it is not certain how many will be acceptable with LNI's over 0.3. (See Table 1 in the Appendix).

Lassa fever cases

The identification of cases of LF increases knowledge of the clinical course of the disease, affords opportunities for therapeutic trials, and indentifies potential donors of LFIP. For the most part this activity was confined to CLH and PH during the year.

The diagnosis of LF is made by the serological testing of febrile patients and by attempts at viral isolation. Again, because of the limited capability for virus isolation at USAMRIID during the year none of the patients at PH were tested virologically, and virus isolation has been attempted to date in only a fraction of the patients at CLH.

As noted previously (5) the diagnosis of LF depends upon the isolation of LV from the serum, or by seroconversion or a fourfold rise in antibody titers in paired specimens by means of the IFA technique. In some cases in which a single specimen is obtained the diagnosis of "Possible LF" (PLF) is made if IFA titers are 1:64 or higher.

During the period through April 30, 1986, 120 febrile patients at CLH were tested serologically for evidence of LF; in 73 of these, virus isolation was attempted as well. In all, 13 cases of LF were diagnosed by virus isolation and 11 by serological criteria; an additional five were considered to be PLF cases because of relatively high LVA titers. The incidence of LF among febrile patients was 0.20, 0.24 if Possible LF was included as well. (See Table 2 in the Appendix)

At PH 252 patients were tested serologically through May 15, 1986. Seven cases of LF and three of PLF were diagnosed for incidences of 0.03 and 0.04 of LF and combined LF and PLF, respectively, among those tested.

Previous work (14) has demonstrated that when both virus isolation and serological testing are performed on febrile patients, serological testing alone will detect 73% of LF and PLF cases. It may be conjectured that if virus isolation had been attempted on all patients of this series at CLH, 16 cases of LF and PLF would have been identified in the period following November, 1955, with a total of 33 and an incidence of 0.28 for the whole 10 months of testing. Similarly, if virus isolation had been attempted at PH, 14 patients with LF would likely have been identified there for an incidence of 0.06.

Passive Immunotherapy

Through March, 1986, LFIP was administered to nine patients at CLH. In one, both virological and serological tests were negative for evidence of LF. In two serological tests were negative and virus isolation has not yet been attempted; the diagnosis of the illnesses of these patients is not yet clear.

Six of the plasma recipients at CLH were confirmed to have had LF, five by virus isolation and one by seroconversion. Four were pregnant; of them, three died. One had received plasma on the 13th day of illness, considered to be too late for LFIP to be effective. (8). Another adult woman, who was not gravid, and a six-month old child survived. All LFIP units were from donors who had previously been found to have relatively high LNI's, and were thus likely therapeutically effective, though recent LNI levels had not been determined.

During the same period six suspected LF patients were treated with plasma at PH. Subsequent serological tests did not confirm the diagnosis of LF in any, and virus isolation has not been attempted in them so far. Two were found to be bacteremic by blood cultures started before plasma infusion but not reported until afterward; of these, one died. None of the other four expired; one was a pregnant woman, another, a non-gravid adult woman, and two were children. Again, no tests for LNI's had been done recently on samples of the donors' plasma.

The small group of patients treated does not permit the drawing of any conclusions. They will eventually form a part of a larger series of patients treated by passive immunotherapy from whose experience some conclusions may be possible.

Conclusion

Plasmapheresis continues in the Liberian hospitals, and during the year permitted the collection of more LFIP than ever before. New donors are being recruited as cases of LF are discovered among febrile hospital patients. An adequate supply of convalescent plasma adequate for the needs of USAMRIID as well as for therapeutic trials in Liberia seems assured for the future.

Passive immnotherapy, as well as the trial of ribavirin among LF patients, using change in levels of viremia as the criterion for evaluation of results, await the more rapid and accurate diagnosis of LF which is likely to accompany the use of the ELISA test for viral antigen. It is expected that this technique will be field-tested, and it is hoped, come into regular use during the coming year.

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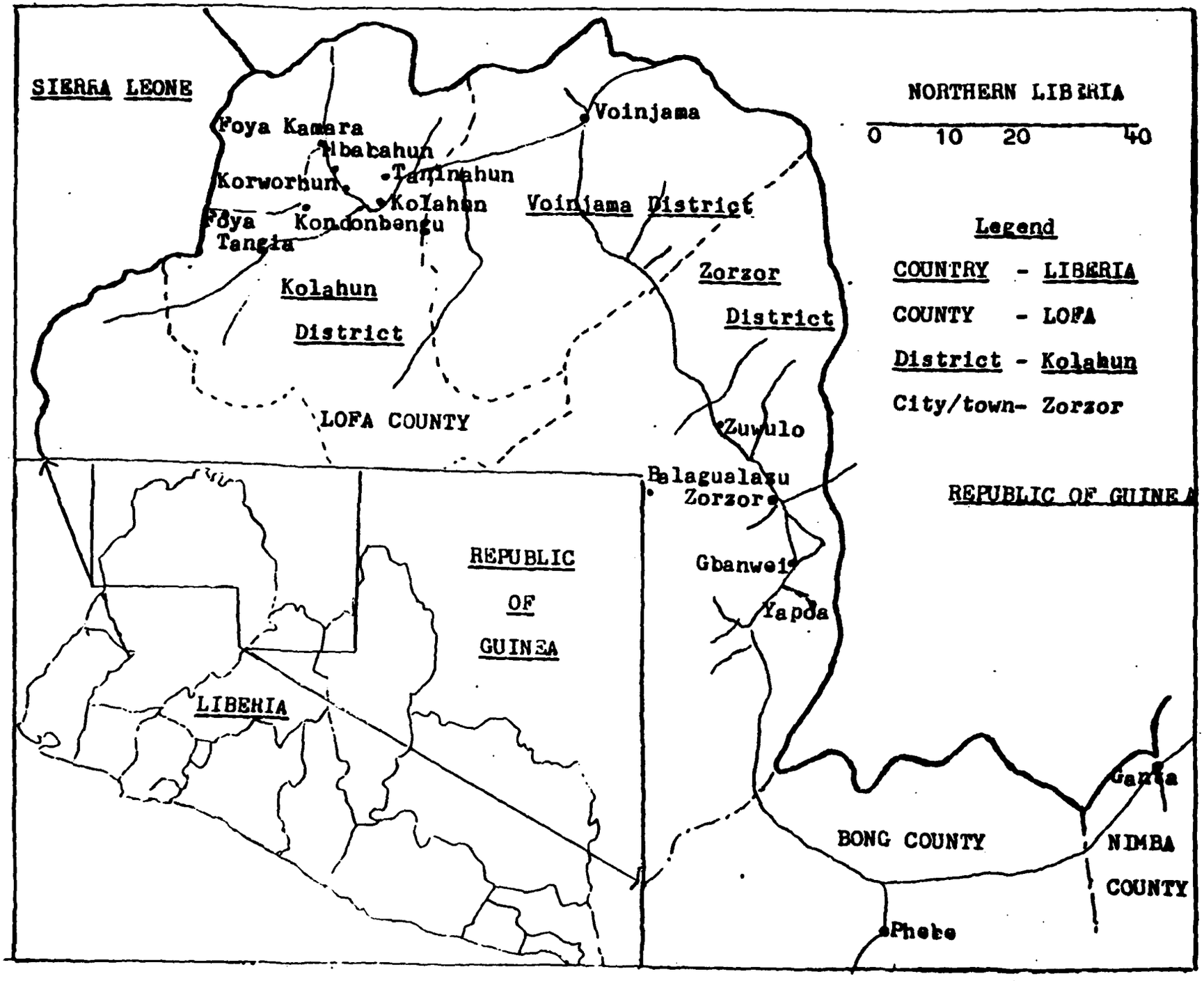

MAP -- NORTHERN LIBERIA

Appendix Tables.

Table 1. Lassa fever immune plasma units collected July 1985 - June 1986.

Donor	Date of illness	Date of donation	IFA titer*	LNI# Jos	LNI# Mac	Number of units Collected	Number of units USAMRIID**
DaBa	11/82	5/12/86	16	1.6	2.2	2	2
		10/7/85	8			2	
YaBu	1/85	6/24/86	?			2	2
GoCo	3/85	10/30/85	64	2.3	2.8	2	
		4/23/86	32	2.2	2.2	2	1
		6/20/86	16			2	2
HeCo	2/85	4/23/86	32			2	1
		6/21/86	8			1	1
OlCo	4/85	10/29/85	256	3.1+	3.6+	2	1
		4/23/86	128	3.1	3.7+	2	1
		6/20/86	64			2	2
DaDo	04/77	10/7/85	?	0.5	0.5	2	1
		3/20/86	-			2	1
		5/14/86	8	0.7	0.6	2	2
MaFa		12/5/85	-	0.3	0.3	2	2
		4/24/86	-			2	1
		6/20/86	-			2	2
KeFl	1983	8/6/85	32	1.0	1.8	2	2
		10/8/85		1.2	1.8	2	1
		12/2/85	32	1.9	2.1	2	2
		3/19/86	16	1.6	1.9	2	1
		5/13/86	8	1.5	2.3	2	2
		6/25/86	16			2	2
LoFl	01/82	5/14/86	8	1.6	1.9	2	2
		6/24/86	4			2	2
JoGa	1976	5/14/86		1.4	1.3	2	2
KlGa	1982	8/8/85	-	0.3	1.5	2	2
		3/20/86	8			2	2
JoHo	3/85	6/24/86	4			2	2

Table 1 (cont)

Donor	Date of illness	Date of donation	IFA titer*	LNI# Jos	LNI# Mac	Number of units Collected	Number of units USAMRIID**
DaJa	10/84	10/7/85	4			2	
		12/3/85	?			2	
		5/12/86	8	0.6	0.5	2	2
IrJo	02/83	5/13/86	64	3.1+	3.7+	2	2
BoKa	10/82	8/8/85	4	0.9	1.3	2	2
		12/2/85	4	1.5	1.7	2	1
		3/19/86	8	1.9	1.7	2	1
		5/12/86	8	2.1	2.6	2	2
		6/24/86	8			2	2
JohKe	3/84	10/8/85	8			2	
		5/14/86	4	1.4	1.6	2	2
MuKe	9/84	6/24/86	-			2	2
JoKo		10/30/85	-			2	1
		4/24/86	4	0.3	0.4	2	1
GaKo	10/83	10/8/85	8	3.1+	3.6+	2	2
GaKoII	7/83	10/8/85	-	0.6	1.1	2	
YaKo	10/81	10/7/85	8	2.5	2.1	2	1
		12/4/85	8			1	
		3/19/86	8			2	2
		6/25/86	8			2	2
DaKo	10/81	8/6/85	-	3.1+	3.6+	2	2
KeKo	7/82	10/8/85	16	0.0		2	1
SoKw		10/31/85	-	0.4	0.4	2	1
		4/24/86	?			2	
KaMa	03/83	8/6/85	8	3.1+	2.5	2	2
		12/3/85	16	3.1+	3.6+	2	1
		3/19/96	8			2	2
		5/12/86	16	3.1+	3.4	2	2
		6/24/86	16			2	2
NoMa	11/82	5/13/86	8	3.1+	2.6	2	2
		6/25/86	16			2	2

Table 1 (cont)

Donor	Date of illness	Date of donation	IFA titer*	LNI# Jos	LNI# Mac	Number of units Collected	Number of units USAMRIID**
JoMi		10/30/85		1.6	1.0	2	1
		4/24/86	8			2	1
		6/21/86	16			2	2
JaMo	?	8/6/85	?	0.0	0.0	2	1
		12/2/85	4	1.4	0.6	2	2
		5/12/86	8	0.3	0.6	2	2
CeMu	04/83	3/20/86	128			2	2
DaMu		10/29/85	-	0.2	0.0	2	1
		4/23/86	?	0.3	0.2	2	1
		6/20/86	-			2	2
JaMu	5/81	10/31/85	?	0.3	0.1	2	1
SaPa		10/29/85	-	0.2	0.1	2	1
		4/23/86	?	0.3	0.1	2	1
		7/25/86	4			2	2
JoPe		12/5/85	8	1.0	0.8	2	2
ErRi	6/84	8/9/85		1.0	1.7	2	2
		10/7/85	128	1.0	1.2	2	1
		12/2/85	32	2.5	0.9	2	2
		6/25/86	128			2	2
YoSe	3/85	8/6/85	32	1.8	1.7	2	1
		10/8/85	32			1	
DaSu	01/83	10/7/85	32	0.4	0.5	2	2
		3/19/86	8	0.6	0.8	2	1
		5/12/86	16	0.6	0.8	2	2
YaTa	9/92	8/8/85	8	0.6	1.4	2	2
JoTo		10/29/85	8	1.1	1.0	2	1
		4/24/86	8			2	1
		6/20/86	4			2	2
BeTo	2/84	8/6/85	16	1.4	1.7	2	2
		12/3/85	16			2	1
		5/13/86	32	3.1+	3.7+	2	2
		6/25/86	128			2	2

Table 1 (cont)

Donor	Date of illness	Date of donation	IFA titer*	LNI# Jos	LNI# Mac	Number of units Collected	Number of units USAMRIID**
DaTo@	03/83	8/6/85	4			2	
		10/7/85	8			2	
		12/3/85	8			2	
		3/19/86	8			2	
NoTo	1984	8/8/85	32	3.1+	3.6+	2	2
		12/3/85	32	3.1+	3.6+	2	1
		5/13/86	16	3.1+	3.7+	2	2
		6/25/86	16			2	2
MaTu		4/24/86				2	1
CaVa	6/81	8/8/85	8	1.8	1.5	2	2
		5/13/86	8	2.0	1.9	2	2
YaVa	02/82	8/8/85		0.9	1.1	2	2
		12/3/85	8	1.3	1.1	2	1
		5/13/86	8	1.0	0.8	2	2
MaVa		10/30/85	?			2	1
		6/20/86	?	0.5	0.9	2	2
BeVa	8/78	10/7/85	?	2.6	0.7	2	1
		12/2/85	-	0.8	1.0	2	2
		3/19/86	?	1.0	0.9	2	1
		6/24/86	?			2	2
JoVa@	1976	10/29/85	?	0.6	0.6	2	1
KlVe	07/83	8/6/85	4	0.9	1.5	2	2
		10/8/85	8	0.9	1.5	2	1
		12/2/85	16	1.5	1.6	2	2
		3/20/86	16			2	1
		5/13/86	4	1.2	1.1	2	2
		6/25/86	32			2	2
RaVe	12/82	10/8/85		0.0	0.1	2	1
		12/2/85	-	0.1	0.8	2	2
		3/20/86				2	1
		5/13/86	?	0.4	0.2	2	2
		6/25/86				2	2

Table 1 (conc)

Donor	Date of illness	Date of donation	IFA titer*	LNI# Jos	LNI# Mac	Number of units Collected	Number of units USAMRIID**
MoWo	04/77	8/6/85	4	2.0	3.6+	2	2
		12/2/85	64	3.1+	3.6+	2	2
		5/12/86	32	3.1+	3.6+	2	2
		6/24/86	32			2	2
MaZa	07/82	5/14/86	16	0.9	0.7	2	2
	Total					255	189

* Expressed as reciprocals of indirect fluorescent antibody titers. Tests performed at the LIBR. Reactivity of reagent varies from batch to batch, and comparisons of values must be made with caution. Not all tests completed at the time of preparation of the Annual Summary Report.

Log Neutralization Index; Josiah (Jos) and Macenta (Mac) strains of Lassa virus used as reagents. Tests performed at USAMRIID.

** In general, specimens were forwarded to USAMRIID which showed an LNI of at least 0.3. Some specimens forwarded before LNI had been determined.

@ Found to be HBsAg positive after LFIP units had been forwarded to USAMRIID.

Table 2. Patients with Lassa fever, Curran Lutheran Hospital (CLH) and Phebe Hospital (PH), July 1, 1985 - April 30, 1986.

Hospital	No tested	Lassa Fever			Possible LF (High IFA titers	Total, LF and possible LF(Rate)	Other IFA pos.
		Virus Isolation	Seroconversion	Total (Rate)			
CLH							
7/1/85-11/30/85	73	13*	4	17 (0.23)		17 (0.23)	5
12/1/85-4/30/86	47	#	7	7 (0.15)	5	12 (0.26)	1
Total, CLH	120	13*	11	24 (0.20)	5	29 (0.24)	6
PH							
7/1/85-5/15/86	252	#	7	7 (0.03)	3	10 (0.04)	19

* Virus isolation attempted in specimens from CLH through 11/30/85

Virus isolation not performed.

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